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Rearing Lygus hesperus in the Laboratory

ABSTRACT

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A culture of Lygus hesperus Knight has been maintained in the laboratory on the Debolt Lygus diet for 5 years. A mechanized process of making various sized empty packets and the adaptation of an inexpensive pump for uniformly filling them have increased efficiency in rearing this insect. An oviposition packet has been developed to provide highly visible eggs. This report includes improvements in techniques, simplified diet preparation, egg collection, and adult handling. The average production of adults per rearing carton has increased from 267 in 1981 to 569 in 1984. The cost per thousand of adults reared on the Debolt diet was $2.82 and on beans $19.94 or seven times greater.

KEYWORDS: Artificial diet, Debolt Lygus diet pump, heat-sealed diet packets, Lygus hesperus, oviposition packets, packet machine, Patana diet packets, rearing.

CONTENTS

Diet preparation, 1

Packet production and use, 2
- Diet packets, 2
- Filling diet packets, 3
- Filling oviposition packets, 4

Rearing operation, 4
- Adult oviposition cages, 4
- Egg handling, 5
- Nymphal rearing, 6
- Adult production, 7
- Cost comparison, 8

Summary, 8

Literature cited, 9

REARING LYGUS HESPERUS IN THE LABORATORY

By Raymond Patana and Jack W. Debolt 1/

Cultures of Lygus hesperus Knight at the Tucson laboratory have been maintained and supplemented with field collections since 1959 or 1960. The cultures were usually reared on green beans (Beards and Leigh 1960, Bottger 1966) or green beans supplemented with heat-killed larvae of the beet armyworm (Spodoptera exigua (Hübner)) (Bryan et al. 1976). The use of green beans required extensive handling and labor to maintain even small colonies, as is generally true with a fresh food source. The scarcity of fresh beans at times overshadowed even their occasional high cost. Since the combination of availability and price often necessitated using whatever beans were available, there was no way to control the quality of food for the insects.

Debolt (1982) reported the first successful artificial diet for rearing successive generations of L. hesperus. Patana (1982) described a heat-sealed disposable packet for feeding the diet to the insects and for use as an oviposition substrate. Debolt and Patana (1985) reported using a dry mineral mix and an improvement in rearing and oviposition cages. Further rearing improvements have been made to increase Lygus production to meet requirements.

This report includes new, currently used equipment, modified mixing procedures, and a cost comparison between the Debolt diet and green beans. Also, various sizes of diet packets and a new oviposition packet are described. No change in the nutritional makeup of the diet was intended, only to simplify and streamline operations and procedures. When any changes were made, they were first tested using the original diet as a comparison standard.

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DIET PREPARATION

The following ingredients are added to 2200 ml of tap or demineralized water in a 3.79-liter blender and mixed for 2 to 3 minutes at high speed:

	Grams
Beans (lima, dry ground)	100
Wheat germ	100
Casein hydrolysate	40
Sucrose	60
Tween 80	2
Cholesterol	1
Ribonucleic acid	1
Lecithin (soybean with oil)	1
Linoleic acid (75 percent)	1

While the ingredients are being mixed, add 6.4 g of Debolt Lygus salt mixture (prepared as shown below) and 12 g of Gelcarin. Mix for 2 minutes, pour into an autoclave container, and autoclave for 20 minutes at 121°C.

Ingredients for the Debolt salt mixture are weighed and mixed as follows in a ball mill and stored in a refrigerator until needed (Debolt and Patana 1985):

	Grams
Sodium phosphate	48
Potassium phosphate	96
Sodium chloride	12
Magnesium sulfate	30
Zinc sulfate	1.32
Cobalt chloride	1.65
Manganese sulfate	1.5
Copper sulfate	.3

Cool the mixture before adding the final ingredients. Originally Debolt (1982) reported cooling to 70°C and then adding

the remaining ingredients. This practice has been changed somewhat. Normally the autoclaved part of the diet is cooled, refrigerated, and finished on the following day. There is a wide temperature range (15°-70°) in which the remaining ingredients can be added without affecting the quality of the diet. Remove the diet from the refrigerator and allow to warm to room temperature or heat to 25° in a water bath. Transfer the diet from the autoclave container to a sterile 3.79-liter blender and mix thoroughly while adding the following ingredients:

Formaldehyde	2 ml
Streptomycin sulfate	.3 g
Aureomycin	1.0 g

Mix for about 2 minutes.

In a sterile household blender container (1 liter), add the following ingredients in the order listed and mix thoroughly for about 1 minute:

Water (tap)	200 ml
Niacin (nicotinic acid)	1 g
PABA (p-aminobenzoic acid)	1 g
Vanderzant Vitamin Modification Mixture for Insects	20 g
Chicken eggs (large, 9 eggs)	approx. 430 ml

Add this egg-vitamin mixture to the other ingredients in the large blender and mix for about 1 minute. The diet is then ready to be dispensed into diet packets for use in feeding.

The mixing procedure was simplified into two operations--mixing before and after autoclaving. Using this method it is now possible to mix the first ingredients, autoclave them, and finish the diet in 1 day. This can be done by quickly cooling the autoclaved diet components in an ice bath or a freezer and then finishing the mix. Other modifications that have been adopted are using untoasted wheat germ and nine large eggs per batch of diet. The untoasted wheat germ is about 2-1/2 times less expensive than the vacuum-packet toasted wheat germ. Using nine eggs per batch eliminates wasting any excess volume of eggs. Originally the diet was centrifuged and only the liquid part was used. The diet now can be used as is, and thus the additional step of centrifuging is eliminated.

PACKET PRODUCTION AND USE

Diet packets

Diet packets were described by Patana (1982). The empty diet packets were made with a household heat sealer that would bond polyethylene film (0.5 mil thickness) with 10.2-cm-wide Parafilm at 14.24-cm intervals and form a continuous roll of packets sealed on two sides. A third side was then sealed, and the packets were separated by cutting through the middle of the cross seals. This formed a packet sealed on three sides. Liquid diet was then placed inside the packet and the fourth side sealed. After removal of the waxed-paper backing from the Parafilm by soaking, the packets were ready for use.

In late 1982, specifications were drawn up for a commercially manufactured sealer. A machine was designated and manufactured by Vertrod Corporation (2037 Utica Ave., Brooklyn, NY 11234) to seal packets on three sides using thermal impulse sealing. The machine seals the Parafilm and polyethylene films in an L-shape, the length of the vertical or cross seal is 10.2 cm, and its horizontal part can seal up to 15.24 cm. The width of each seal is 0.953 cm. With this machine, a packet can be sealed on three sides in one operation by connecting the L's to form LLLL (the bottom line forming a continuous seal)

(fig. 1). The length of the packet is determined by the distance between the vertical lines of the "L's," but the seal can be made at any desired interval between 0 and 15.24 cm. Multiples of 5.08 cm are used because the waxed-paper backing of the Parafilm is lined at these intervals and thus allows for simple alignment on these lines. The sealing jaw of the machine is powered by a compressed air cylinder controlled by an electric foot switch. The polyethylene film and Parafilm are placed on rollers aligned with the sealer jaw. The operator pulls the materials from left to right after making each seal. The three-sided packets are gathered onto a cardboard tube in a continuous roll. Individual packets are made by cutting through the middle of the cross seal with a papercutter.

Figure 1
Using pneumatic heat-sealing device to make packets sealed on three sides.

Filling Diet Packets

The liquid diet is placed in the larger three-sided packets using a plastic gear oil-dispensing pump (Sta-Lube, Inc., P.O. Box 5746, Compton, CA 90224 - Product No. 4344). The amount of diet the pump delivers can be regulated by placing a hose clamp on the exposed part of the piston to adjust the length of the downward piston stroke and thus the desired volume of diet is delivered (fig. 2). For filling 10.16- by 15.24-cm packets, use 20 ml of diet, and for 10.16- by 10.16-cm packets, use 10 ml of diet. For smaller packets (5.08 by 10.16 cm), place the diet in a plastic squeeze bottle and add about 5 ml to each packet.

The procedure for filling the packets consists of opening the packet, dispensing the diet into it, removing most of the air, and sealing the remaining side, using a 'foot' pedal-operated heat sealer (Sealmaster No. 230, Audo Elektro, P.O. Box 3430, 1001 AE Amsterdam, Holland). The diet inside the

Figure 2
Diet pump used to fill diet packet.

packet is spread and the packet placed in lukewarm water to soak the waxed-paper backing. The backing paper is removed from the packet, and the packets are then stacked four to five layers high on a tray and refrigerated until needed. The diet can be stored up to 2 weeks when held at about 5.0°C. When it is stored for even 2 to 3 days, a certain amount of separation occurs between the liquid and solid components. The separated diet can be recombined by gently shaking the individual packets before use.

Filling Oviposition Packets

Specially filled packets have been developed for L. hesperus oviposition. The 10.16-cm-square packets are used. They are filled with warm Gelcarin solution (50°C, 12.5 g/L water). The dry Gelcarin is blended with hot tap water (ca. 60°), and the resulting solution is autoclaved for 20 minutes. After the solution is removed from the autoclave, it is held at 66° to keep it liquid. To fill the oviposition packets, place the solution in a water bath and cool to 50°. This temperature allows the solution to remain fluid so that it can be dispensed with the same type of pump as described for dispensing the diet. It is important that the solution be cooled to 50° before dispensing or it will partially melt and distort the pump. Also if it is used too hot, it will partially melt the Parafilm and bond it to the waxed-paper backing so that it is almost impossible to remove the backing. To fill the packet, open it, pump the Gelcarin solution into it, remove all possible air from inside the packet, heat-seal the remaining side, and place the packet on a flat surface with the Parafilm side down. When the Gelcarin cools, it gels, retains its flat shape, and provides a better surface for oviposition.

REARING OPERATION

Adult Oviposition Cages

The adult oviposition cages are made from clear plastic storage boxes, 26.7 by 34.2 by 8.6 cm, as described by Debolt and Patana (1985). A section of the bottom of the box, 22.9 by 29.9 cm, is cut out and replaced with aluminum window screen glued in place with a hot-melt glue gun. A 2.5-cm hole is drilled into one end of the cage for introducing insects, and the hole is closed with a rubber stopper (fig. 3). Autoclaved industrial brown hand towels or scrap typing paper cut into 5.3-cm-wide strips is crumpled and placed in the cage to provide isolated resting spaces. The cage is then closed with a lid held in place with tape. It is inverted so that the screened side is up.

Two thousand recently enclosed L. hesperus adults are placed in the cage, and three diet packets, 10.16 by 15.24 cm, are placed on the cage with the Parafilm side down. The onset of oviposition can be determined by checking the Parafilm surface of the diet packets on the newer cages. A simple way to determine wheth-

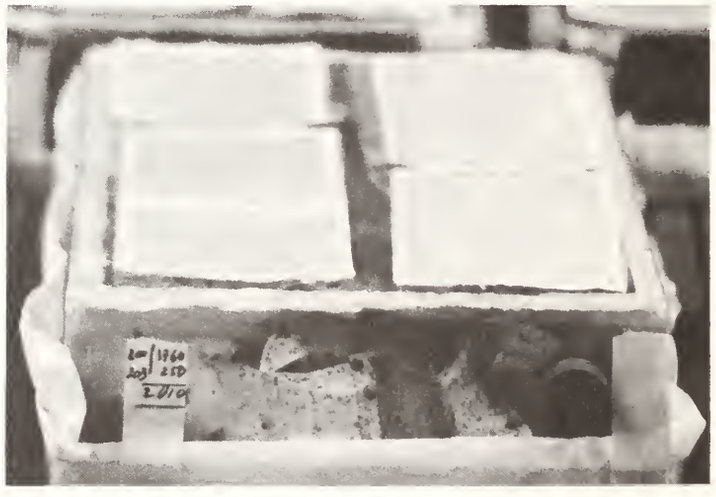

Figure 3
Oviposition cage with diet and oviposition packets.

er eggs are present is to lightly rub the index finger along the Parafilm surface. If there are eggs, the egg caps can be felt on the otherwise smooth surface. After the eggs are detected, three Gelcarin packets are placed on the cage in addition to the three diet packets. The diet and Gelcarin packets on the cages are changed daily. The diet packets with eggs are saved and used for parasite rearing. The eggs in the packets containing only Gelcarin are very visible. The Gelcarin packets are used for continuing the culture, for parasite production, and for research purposes.

Originally when the first Gelcarin packets were developed in 1981, egg counts were made. The packets selected for these counts generally had a lower egg density and wider separation between the eggs to ease the difficulty of counting. Based on a total of 67 packets over a 5-week period, the average number of eggs per packet was 1,897. Overall production levels have increased considerably since then, but this figure is still used as a basis to estimate eggs. In maintaining the culture and providing eggs for parasite exposure, the packets with the heaviest eggs are assumed to have about 4,000 eggs, with a gradation down to sparser densities (but still with a uniform egg distribution) at 2,000 eggs per packet. Because of the efficiency of the overall rearing system, it is possible to select the cages with the most active insects so that most of the oviposition packets contain near the maximum number of eggs. Based on visual comparisons of packets with 2,000 eggs and those with higher densities, the 4,000 count may be slightly low.

Insects are kept in the oviposition cages for 21 days. If held longer, egg production declines.

Egg Handling

The number of packets saved to continue the culture depends on the production level desired. Six heavy egg packets are saved daily to maintain a culture of 1,500 new adults per day. Of these, only three halves or thirds of packets are used for production and the remainder are utilized for parasite rearing. The extra eggs also provide a way to increase production on short notice. The procedure for handling eggs remains essentially the same regardless of production levels. Trim the egg packets around three sides, lift the polyethylene film, immerse the Parafilm in 40°C water, and allow to soak for a few minutes. The Lygus female inserts the egg through the Parafilm layer and into the solidified Gelcarin solution inside the packet. Only the cap of the egg is exposed on the outside of the Parafilm, with the body of the egg extending into the Gelcarin layer inside. Soaking in the warm water dilutes the Gelcarin around the eggs and thus frees them from the Gelcarin and leaves the eggs in the Parafilm. Using forceps, grasp the opened packets by the polyethylene flap, shake gently in the water, and lift out. The Gelcarin layer separates from the Parafilm and leaves all but very few eggs embedded in the Parafilm. Place the opened packets in a 0.03 percent sodium hypochlorite solution for about 2 minutes, then rinse with water. Fold back the polyethylene film in place over the eggs, and place the packets in a plastic box for development.

For holding 6-12 packets, use a plastic shoe box, 31.1 by 16.5 by 8.9 cm (Style No. SB-100, Sterling Products Co., 1689 Oakdale Ave., St. Paul, MN 55118); for 12-36 packets, use a plastic store-all box, 34.3 by 26.7 by 8.9 cm (Style No. UB-200). Line the bottom of the box with a triple-ply disposable towel, 38.1 by 42.9 cm. Place a layer of facial

tissue in the box and then the washed and sterilized packets on the tissue Parafilm side up, so that the caps of the eggs are oriented up. Place the packets in the box wet as they come from the water rinse. Pile them three layers deep, with facial tissue between each layer. The moisture protects the eggs from drying out during development. Cover the egg-packet holding box with a triple-thickness disposable towel, either single or folded double (for a large box or a shoe box), and cover with a lid. Keep the egg boxes at about 60 percent relative humidity and 25°C. Check for moisture and development at 2, 3, and 4 days. If the eggs or the interior of the box is dry, add water with a squeeze bottle. When the box is held at 25°, development can be observed after 4 days under a microscope by the presence of red eyespots in the eggs.

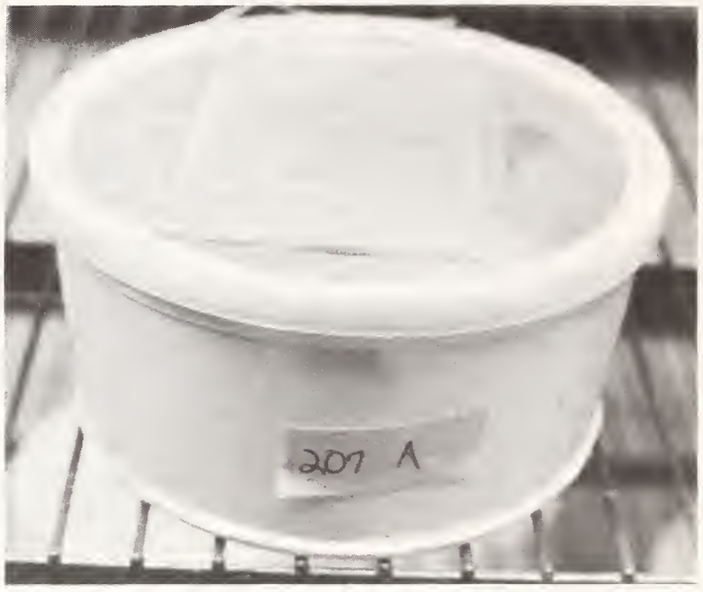

Figure 4
Lygus sp. nymphal rearing container with organdy lid and diet packet.

Nymphal Rearing

Nymphal rearing cartons are made from 3.79-liter plastic tubs and lids (Lily 10 MP and 10M85-3, Lily, Division of Owens-Illinois, Toledo, OH 43666). The bottom 11.4 cm of the tub is cut off, and the opening, about 18 cm in diameter, is then closed with an 18.4-cm-diameter section cut from the center of a lid that is glued in place to form a shortened tub. The cut-out part of the lid is replaced with a piece of nylon organdy glued into place. An equal number of lids are made up with the cut-out section replaced with fine Saran screen glued into place (fig. 4). The organdy lids are used for newly hatched nymphs and the screened lids for larger nymphs.

In setting up nymphal rearing cartons, use 5-day-old eggs and one-half to two-thirds of an egg packet, based on a visual estimate of the number of eggs. This provides about 1,500 eggs. Remove the polyethylene film and place the Parafilm with the eggs in the bottom of the rearing carton with the cap side of the eggs up. Place a 15-cm^2 section of 6-mm mesh hardware cloth screen with each corner bent down 4 cm over the eggs. This provides a protective spacer for the eggs and holds a 15.24-cm diet packet as an additional feeding site for the nymphs. In the space above the diet packet and below the top of the carton, place crumpled 6-cm-wide strips of sterile (autoclaved) brown paper hand towels. The crumpled towels provide hiding and resting places for the nymphs and access for small nymphs to the cage top.

For the first 14 days, use an organdy covered lid on the carton. When held at 25°C and 60-66 percent relative humidity, the eggs hatch in 2-3 days (7-8 days from date of collection). The high humidity is important in nymphal survival (Debolt 1982). Change the packets every other day until the 10th day, when the rearing carton is moved into the nymphal rearing room maintained at 25°

and 20-50 percent relative humidity, and then change the diet packets daily. On the 15th day, replace the lid with a Saran screen-covered lid. The Saran screen is not used for smaller nymphs because they can escape through it. When used with later nymphal stages, the Saran allows a greater air exchange to the Parafilm surface. This permits the feeding punctures in a Parafilm to dry and seal and thus lessens leakage. Occasionally some leakage occurs, increasing during high humidity and favoring mold and yeast development. In the Tucson area, this is less of a problem than it would be in areas of higher humidity. This leakage probably occurs when high numbers of nymphs feed on a particular area and thus prevent feeding punctures from sealing. Overcrowding of nymphs in the rearing containers also increases this problem.

At 25°C, the nymphs become adults after 21 days (egg collection - adult). At this time, aspirate the adults from the rearing cartons, count them, and place them in oviposition cages. The main section of the aspirator consists of a 2.2-cm-diameter clear plastic tube 30 cm long, with an inside diameter of 1.9 cm. A fine nylon screen or nylon organdy is placed over one end of the tube, and it is slipped into the large end of a nylon baster tube (Ekco Nylon Baster No. 00378, Ekco Housewares Co., Franklin Park, IL 60131) with the bulb removed. The small end of the baster tube is connected to a vacuum source. Another baster tube is placed on the open end of the clear plastic tube to provide a reduced diameter opening.

The Lygus rearing cartons are opened in a light box made from a cardboard box, 61 cm long, 42 cm wide, and 40 cm deep, with the bottom removed and with aluminum window screen. A fluorescent light fixture with 2-15 watt black light bulbs is placed behind the light box. The Lygus adults are attracted to the black light and can be easily aspirated off the screen and counted (fig. 5). The aspirator will hold 200 to 300 adults without injuring them, but in general use, empty the tube after 200 are collected. To empty the aspirator, slip off the front baster tube, put the open end of the tube through the 2.5-cm opening of the oviposition cage, shut off the vacuum, and disconnect the hose. Blow gently on the opposite end of the tube and thereby force the Lygus adults out into the cage.

Adult Production

The total annual laboratory production of L. hesperus adults (table 1) did not increase greatly, but the increase in average number of adults per carton indicates improved efficiency. The number of egg packets used per carton decreased from three in 1981 to one packet in 1982, and to one-half or less in 1983 and 1984.

Figure 5
Aspirating adult Lygus hesperus from screen box, with black light on other side of screen.

Table 1
Weekly and annual laboratory production of *Lygus hesperus* adults on Debolt *Lygus* diet, 1981 to mid-1984[1/]

Measure	1981	1982	1983	1984
Weekly (average)	Number	Number	Number	Number
Adults.....	9,839	9,948	13,169	13,861
Adults per carton ± S.D...	285±183.7	472±70.3	397±69.9	551±174.0
Annual				
Adults (total).....	511,614	517,285	684,761	346,519
Adults per carton (average)	267.3	474.6	508.7	569.0
Cartons used (total)	1,914	1,090	1,346	609

[1/]First 25 weeks.

Cost Comparison

The cost of rearing *L. hesperus* on the Debolt *Lygus* diet versus green beans was compared to determine the differences. Since it was not feasible to run a parallel study, the comparison was made between 2 different years. The data for rearing on the diet and on green beans were obtained, respectively, from the first week in August through October of 1981 and for a similar period in 1979. The average price for beans bought from August through October 1979 was calculated to be $0.45 per pound, with a range of $0.37 to $0.54.

The cost for both methods was calculated on a weekly basis. The actual weekly cost of the diet, including oviposition packet materials, and the beans was respectively, $44.52 and $47.56. However, when the cost was determined on adult insects-per-thousand-basis, there was a considerable difference--$2.82 for the diet and materials as opposed to $19.94 for the green beans, or about seven times greater.

These costs for both methods are exclusive of labor. The amount of labor required for each is similar and increases proportionally within certain production levels.

SUMMARY

The modification and improvement in the laboratory rearing of *L. hesperus* by using simplified mixing procedures and new equipment have resulted in a more efficient production system. The diet is now mixed in two stages--before and after autoclaving. By using a dry mineral mix and a commercially available vitamin mix, a great deal of measuring

has been eliminated. The diet can now be used as is after final mixing and thus centrifuging is omitted. The adoption of the mechanical packet maker provides an efficient method of producing uniform packets for both feeding and oviposition. The development of the Gelcarin oviposition packet is effective in providing highly visible eggs.

The use of the diet pump is a simple method for uniformly filling both types of packets. By using cheaper ingredients and fewer mixing steps, the cost of rearing has been lowered.

The utilization of the new equipment is a step toward automation of the packet-making process.

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